

What is Claimed is:

1. A plasmid wherein two restriction enzyme recognition sites into which a T-vector can be cloned are introduced at the downstream of a promoter of a vector
5 that is constantly expressed at high levels regardless of the kind of a host cell, whereby the plasmid functions as both the T-vector and an expression vector and has the property of allowing the expression of a target gene to be examined only by one-step T-vector cloning.
- 10 2. The plasmid according to claim 1, wherein the restriction enzyme recognition sites into which the T-vector can be cloned is selected from the group consisting of *Hph*I, *Mbo*II, *Asp*EI and *Xcm*I, and a polynucleotide is inserted between the two restriction enzyme recognition sites.
- 15 3. The plasmid according to claim 2, wherein a nucleotide having thymine bases at both 3'-ends of the removal position of the inserted polynucleotide is exposed, when the plasmid is cut with the restriction enzymes.
4. The plasmid according to claim 1, wherein the constitutive high-level
20 expression vector is pHCE.
5. A plasmid (pHCE-FOREX) functioning as both a T-vector and an expression vector, wherein two *Asp*EI restriction enzyme recognition sites are introduced at the downstream of the HCE promoter of the pHCE vector, and a polynucleotide
25 having *Asp*EI restriction enzyme recognition sites at its both ends is inserted between the two *Asp*EI restriction enzyme recognition sites.
6. A constitutive high-level expression T-vector (pHCE-FOREX-T), which is obtained by digesting the plasmid pHCE-FOREX of claim 5 with an *Asp*EI
30 restriction enzyme, to remove the polynucleotide having *Asp*EI restriction enzyme

recognition sites at its both ends, and in which a nucleotide having thymine bases at both 3'-ends of the removal position of the polynucleotide is exposed.

7. A method for producing a plasmid (pHCE-FOREX) functioning as both a T-
5 vector and an expression vector, the method comprising the steps of:
- (a) constructing pHCE-M1 which the restriction enzyme recognition sites were removed by inducing point mutation in *AspEI* restriction enzyme recognition sites in a pHCE vector;
 - (b) constructing pHCE-M2 by introducing two *AspEI* restriction enzyme
10 recognition sites into the downstream of the HCE promoter of the pHCE-M1 vector by PCR using primers containing the *AspEI* restriction enzyme recognition sites; and
 - (c) inserting a polynucleotide having *AspEI* restriction enzyme recognition sites at its both ends, between the two *AspEI* restriction enzyme recognition sites
15 of the pHCE-M2 vector.
8. An expression vector, which is obtained by digesting the plasmid of claim 2 with the restriction enzymes to remove the inserted polynucleotide, and then inserting a gene encoding a target protein, into a position from which the
20 polynucleotide was removed.
9. An expression vector, wherein a gene encoding a target protein is inserted into the constitutive high-level expression T-vector (pHCE-FOREX-T) of claim 6.
- 25 10. The expression vector according to claim 8 or 9, wherein the target protein-encoding gene is a gene amplified by PCR.
11. The expression vector according to claim 8 or 9, wherein the target protein-encoding gene is a PCR product amplified by using a primer having the amino
30 terminal end of ATG, and a primer specific to the base sequence of the gene.

12. The expression vector according to claim 8 or 9, wherein *NdeI* restriction enzyme recognition site is formed in the insertion position of the gene encoding the target protein.
- 5 13. A microorganisms transformed with the expression vector of any one claim among claims 8 to 12.
14. A method for expressing a gene encoding target protein, which comprises
10 culturing the transformed microorganisms of claim 13.
15. An expression vector library, which is prepared by the method comprising the steps of:
- 15 (a) removing the inserted polynucleotide by digesting the plasmid of claim 2 with the restriction enzyme selected from the group consisting of *HphI*, *MboII*, *AspEI* and *XcmI*; and
- (b) inserting the library of various genes into a position from which the polynucleotide was removed.
- 20 16. An expression vector library wherein the library of various genes is inserted into the high-level expression T-vector (pHCE-FOREX-T) of claim 6.
17. A method for determining the cloning of a target gene, the method comprising the steps of:
- 25 (a) transforming microorganisms with the expression vector library of claim 15 or 16; and
- (b) culturing the transformed microorganisms.

18. The method for determining the cloning of a target gene according to claim 17, wherein further comprising the steps of: separating a plasmid after the step (b); and digesting the plasmid with an *NdeI* restriction enzyme.